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Dynamics of Humoral Immunity Indices and of Haematological Variables in Rabbits Immunized with *Chlamydomphila psittaci*

Dynamika wskaźników odporności humoralnej oraz wskaźników hematologicznych u królików immunizowanych *Chlamydomphila psittaci*

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Abstract

Introduction. The research of humoral immunity in infection or immunization with *Chlamydomphila psittaci* in static models showed changes in parameters of this immunity.

Objectives. Evaluation of chosen parameters of humoral immunity in dynamic model in rabbits immunized with *Chlamydomphila psittaci*.

Material and Methods. The studies were performed on rabbits divided into two groups of 10 animals each. The rabbits of first group were immunized with *Chlamydomphila psittaci* – strain 6BC, and the animals of second group were control animals. Blood samples were examined 9 times every 7 days. The activity of myeloperoxidase in polymorphonuclear leukocytes (PMN), amount and activity of lysozyme, total amount of immunoglobulins (Ig), including those of IgG class were estimated in sera of rabbits immunized with *Chlamydomphila psittaci*. Haemoglobin level, content of erythrocytes and leukocytes, blood differential count and titre of *Chlamydia* spp.-specific antibodies were also established by complement-fixation technique.

Results. The results indicate that immunization of rabbits with the bacteria induces with a similar frequency increase or decrease in 2 out of 6 examined parameters of humoral immunity (amount and activity of lysozyme) and rather a decrease than increase in 3 out of 8 examined haematological variables (count of neutrophils, basophils and monocytes). The alterations, mainly those in immune parameters, could be noted 5 weeks before appearance of *Chlamydia* spp.-specific antibodies, which appeared on 49th–56th day of the experiment.

Conclusion. The alterations noted in parameters of humoral immunity, may turn to be useful in determining the condition of macro-organisms in contact with *Chlamydomphila psittaci* (*Adv Clin Exp Med* 2006, 15, 2, 285–291).

Key words: *Chlamydomphila psittaci*, rabbit, immune indices, haematological indices.

Streszczenie

Wprowadzenie. Badania odporności humoralnej w zakażeniu lub immunizacji bakteriami *Chlamydomphila psittaci* w układach statycznych wykazały zmiany wskaźników tej odporności.

Cel pracy. Ocena zmian wybranych parametrów odporności humoralnej w układzie dynamicznym u królików immunizowanych *Chlamydomphila psittaci*.

Material i metody. Badania przeprowadzono na królikach podzielonych na dwie grupy, po 10 zwierząt każda. Króliki z pierwszej grupy immunizowano *Chlamydomphila psittaci* – szczep 6BC, a zwierzęta z drugiej grupy były grupą kontrolną. Krew do badań pobierano 9-krotnie w odstępach 7-dniowych. Oznaczano aktywność mieloperoksydazy w komórkach polimorfonuklearnych (PMN) oraz ilość i aktywność lizozymu, ogólną ilość immunoglobulin (Ig), w tym Ig klasy G, w surowicy u królików immunizowanych *Chlamydomphila psittaci*. Oznaczano także ilość hemoglobiny, liczbę erytrocytów i leukocytów, obraz jakościowy krwi oraz miano swoistych przeciwciał anti-*Chlamydia* sp.

Wyniki. Z opisanych badań wynika, że immunizacja królików tą bakterią powoduje wzrost i spadek występujący z podobną częstotliwością w 2 z 6 badanych wskaźników odporności humoralnej (ilość i aktywność lizozymu) oraz częściej spadek niż wzrost w 3 z 8 badanych wskaźników hematologicznych (liczba neutrofilów, bazofilów i monocytów). Zmiany, głównie wskaźników odpornościowych, stwierdzono 5 tygodni przed pojawieniem się swoistych przeciwciał anti-*Chlamydia* sp., które pojawiły się w 49.–56. dniu doświadczenia.

Wnioski. Zmiany wskaźników odporności humoralnej mogą być przydatne do określania stanu makroorganizmu po kontakcie z *Chlamydomphila psittaci* (*Adv Clin Exp Med* 2006, 15, 2, 285–291).

Słowa kluczowe: *Chlamydomphila psittaci*, królik, wskaźniki odpornościowe, wskaźniki hematologiczne.

The species of *Chlamydomphila* (*Chl.*) *psittaci* belongs to *Chlamydomphila* genus in the family of *Chlamydiaceae* and *Chlamydiales* order [1]. It includes 8 serotypes, pathogenic for humans, many species of birds as well as for cattle, horses, turtles, hares and musk-rats [1].

Studies related to humoral immunity in humans and animals infected or immunized with microbes belonging to *Chlamydomphila* spp. and *Chlamydia* spp. have demonstrated that the conditions augment activities of myeloperoxidase (MPO) [2, 3] and of peroxidase [4] in polymorphonuclear leukocyte (PMN) and mononuclear leukocyte (MN). Also augmented amounts of lysozyme (LZM) [5, 6] and of other lysosomal enzymes (cathepsin D, acid phosphatase, β -glucuronidase) [7] have been noted, as well as those of IFN- α , TNF- α , IL-1, IL-6, IL-12 [8–13]. Immunization of animals with the bacteria has been shown to elevate, although in an irregular fashion, levels of IgG, IgM and IgA class immunoglobulins in serum and in secretions (s-IgA in milk) [14–19]. The data presented in the papers [2–19] have indicated also that only individual parameters of humoral immunity were determined in mammals immunized with *Chlamydia* spp. and *Chlamydomphila* spp. microbes.

Present studies aimed at evaluation of dynamic alterations in indices of specific and non-specific humoral immunity as well as in haematological and serological variables in rabbits (the standard animal in biological studies on humans and animals) immunized with *Chlamydomphila psittaci* – strain 6BC (earlier termed *Chlamydia psittaci* – strain 6BC).

Material and Methods

The studies were performed on 20 mixed breed rabbits weighing each 2.5 kg to 4.0 kg, classified as animals of CV-III group or conventional animals free of pathology [20]. The rabbits were kept in vivarium, in living and zoohygienic conditions consistent with the national standards [20] and were fed full portion LSK chow for rabbits.

Animals of the immunized group (10 rabbits) received intramuscular injection of *Chlamydomphila psittaci* – strain 6BC (termed previously *Chlamydia psittaci* – biotype 3–9) antigen, isolated from a human individual infected from a parrot, of 50 μ g protein/ml, dissolved in 1.0 ml sterile

saline, on the 1st and the 7th day of the experiment. Animals of the control group (10 rabbits) were injected intramuscular in parallel with the same amount of sterile saline.

Blood for tests was sampled on the first day, i.e. before administration of *Chl. psittaci* – in the groups of immunized rabbits and control rabbits and, then, every seven days.

Determination of Humoral Immunity

Activity of MPO in PMN cells was assayed according to the technique of Graham as described by Zawistowski [21], amount (concentration) of LZM in serum was established by plate diffusion technique against *Micrococcus luteus* according to the technique provided by Hankiewicz [22], while activity of LZM was established using the formula provided by Szmigielski [23]. The general immunoglobulin level was estimated according to McEwan [24] and serum IgG class immunoglobulins were estimated by the plate technique against the standards (ICN, USA).

Haematological Tests

Haemoglobin level, red blood cells (RBC) and white blood cells (WBC) levels and differential WBC count were estimated by the standard techniques.

Serological Studies

Presence of *Chlamydia* spp.-specific antibodies in rabbit sera was established by complement fixation technique, against the antigen as the standard. The test was performed as recommended by the manufacturer instruction and complete inhibition of haemolysis (++) at the dilution of 1 : 32 and higher was taken as indicating a positive titre [25].

Analysis of the Results

The immune and haematologic indices were evaluated in respect to the trends on changes in the course of the experiment, comparing results

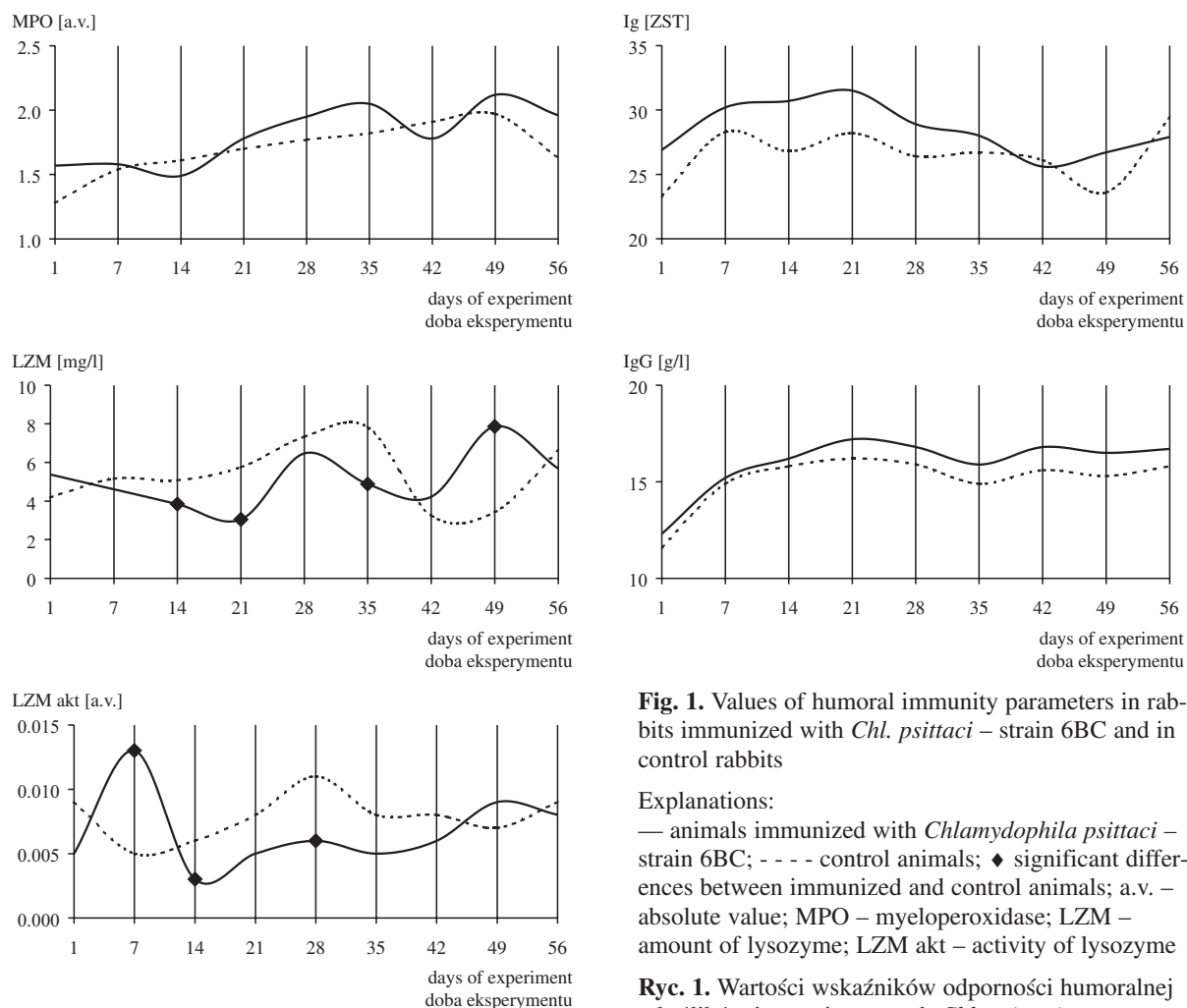


Fig. 1. Values of humoral immunity parameters in rabbits immunized with *Chl. psittaci* – strain 6BC and in control rabbits

Explanations:

— animals immunized with *Chlamydomphila psittaci* – strain 6BC; - - - control animals; ♦ significant differences between immunized and control animals; a.v. – absolute value; MPO – myeloperoxidase; LZM – amount of lysozyme; LZM akt – activity of lysozyme

Ryc. 1. Wartości wskaźników odporności humoralnej u królików immunizowanych *Chl. psittaci* – szczep 6BC i w grupie kontrolnej

Objaśnienia:

— zwierzęta immunizowane *Chlamydomphila psittaci* – szczep 6BC; - - - zwierzęta z grupy kontrolnej; ♦ różnice istotne statystycznie między zwierzętami immunizowanymi a zwierzętami z grupy kontrolnej; a.v. – wartość bezwzględna; MPO – mieloperoksydaza; LZM – ilość lizozymu; LZM akt – aktywność lizozymu

obtained in individual days of the experiment in immunized rabbits to those obtained in control rabbits and employing Student's *t*-test at $p \leq 0.05$ (Figs. 1–3).

Results

In analysis of the five studied immune parameters in rabbits immunized with *Chlamydomphila psittaci* – strain 6BC (Figs. 1–3), growing tendencies were noted even if significant alterations in forms of an increase or a decrease were recorded only in respect to the amount and activity of LZM; they could not be noted in MPO activities and total amounts of immunoglobulins or levels of IgG.

In analysis of MPO activities in the immunized rabbits their values exhibited a slightly increasing trend (Fig. 1), although the alterations were not significant. Amounts of LZM in serum also demonstrated an increasing tendency with extensive variation which proved to be significant. Decreases in the values were noted on the 14th, 21st and 35th day and increase on the 49th day of the studies. Activities of LZM behaved in a similar

fashion but significant alterations in the form of a decrease were noted in 14th day and 28th day while augmented LZM activity was observed on the 7th day (Fig. 1). Total amounts of serum Ig remained on a more less identical level (Fig. 1). On the other hand, IgG class immunoglobulins manifested a slightly growing trend (Fig. 1) but, similarly to total Ig levels, no significant differences were encountered.

Out of the 8 haematological parameters (levels of haemoglobin, RBC, WBC, lymphocytes, neutrophils, eosinophiles, basophiles, monocytes) examined in rabbits immunized with *Chlamydomphila psittaci* – strain 6BC (Figs. 2, 3), significant alterations developed only in levels on neutrophils, basophiles and monocytes. The changes

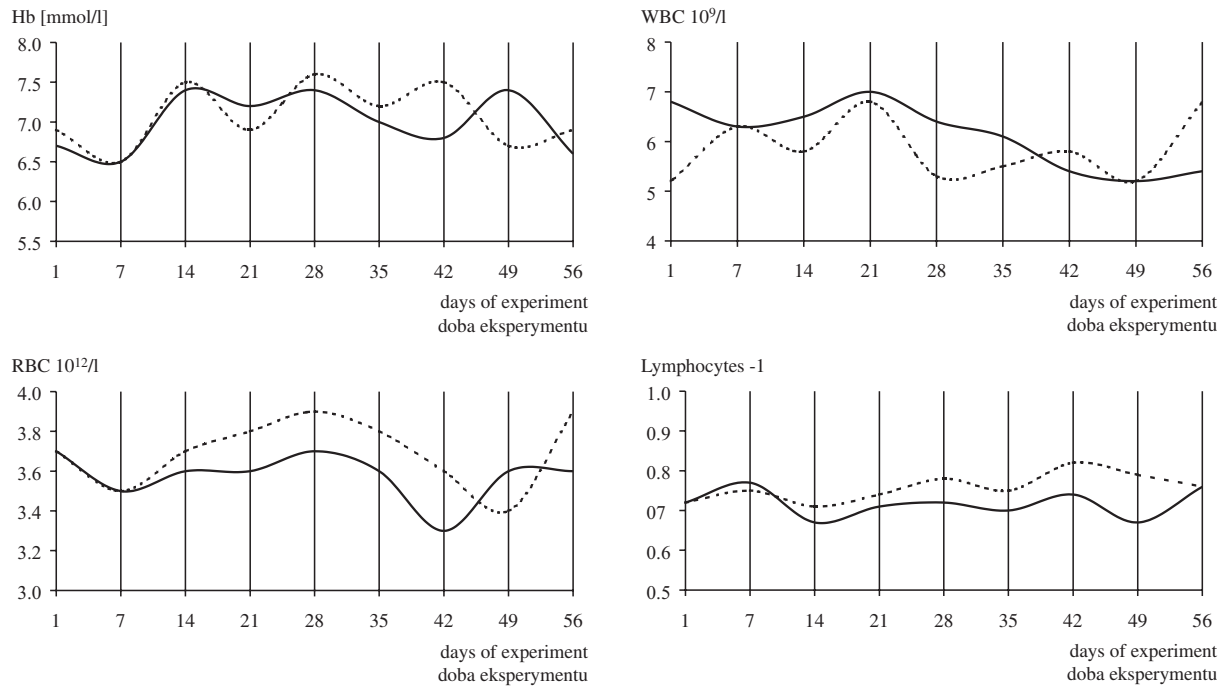


Fig. 2. Haematological indices in rabbits immunized with *Chl. psittaci* – strain 6BC and in control rabbits

Explanations: — animals immunized with *Chlamydomphila psittaci* – strain 6BC; - - - - control animals; ♦ significant differences between immunized and control animals; Hb – haemoglobin; RBC – red blood cells; WBC – white blood cells.

Ryc. 2. Wskaźniki hematologiczne u królików immunizowanych *Chl. psittaci* – szczep 6BC i w grupie kontrolnej

Objaśnienia: — zwierzęta immunizowane *Chlamydomphila psittaci* – szczep 6BC; - - - - zwierzęta z grupy kontrolnej; ♦ różnice istotne statystycznie między zwierzętami immunizowanymi a zwierzętami z grupy kontrolnej; a.v. – wartość bezwzględna; Hb – hemoglobina; RBC – erytrocyty; WBC – leukocyty.

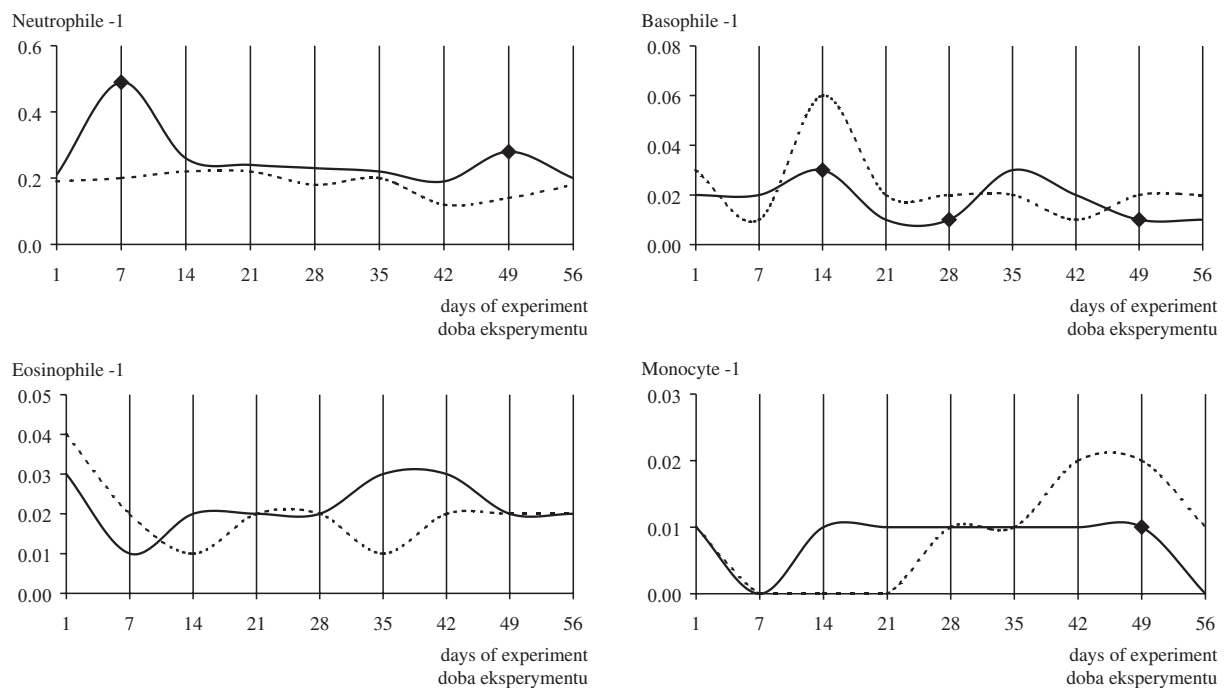


Fig. 3. Haematological indices in rabbits immunized with *Chl. psittaci* – strain 6BC and in control rabbits

Explanations: — animals immunized with *Chlamydomphila psittaci* – strain 6BC; - - - - control animals; ♦ significant differences between immunized and control animals.

Ryc. 3. Wskaźniki hematologiczne u królików immunizowanych *Chl. psittaci* – szczep 6BC i w grupie kontrolnej

Objaśnienia: — zwierzęta immunizowane *Chlamydomphila psittaci* – szczep 6BC; - - - - zwierzęta z grupy kontrolnej; ♦ różnice istotne statystycznie między zwierzętami immunizowanymi a zwierzętami z grupy kontrolnej.

involved an increase in neutrophile content on the 7th and 49th days and decreases in basophile level on the 14th, 28th and 49th days and in monocyte level on the 49th day of observation.

In analysis of serological results the specific antibodies appeared already in the 7th day but dilutions of 1 : 4 and 1 : 8 yielded titres of only 4+ and 1+. On subsequent days, i.e. on the 14th, 21st and 28th days they were detected in most of examined rabbits on dilutions of 1 : 2 and 1 : 4 (4+). On the same days dilutions of 1 : 8, 1 : 16 and 1 : 32 yielded titres of 1+ to 2+. On the 35th day, sera of most rabbits yielded 4+ titres in dilutions of 1 : 2, 1 : 4 and 1 : 8 3+ titres in dilution of 1 : 16 and most frequently 1+ titre in dilutions of 1 : 32. On days 42 to 56, 4+ titre was recorded in all the animals at dilutions of 1 : 2, 1 : 4, 1:8 and 1 : 16. On the remaining dilutions, 1 : 32 inhibition of haemolysis was graded at 1+ to 2+ and in the dilution 1 : 64 respectively at 2+ and 1+.

Discussion

Analysing results of the studies one should note that the absence in significant alterations in MPO activities cannot be unequivocally interpreted due to the lack of analogous studies in rabbits. It should, however, be observed that the result is distinct from those noted in humans infected with *Ch. trachomatis* biotype LGV [3] or *Ch. trachomatis* serotype L₂ [2], in whom augmented activity of MPO was noted in PMN cells [2, 3]. On the other hand, the alterations in the amount (increase on the 49th day, decrease on the 14th, 21st and 35th days) and activity of LZM (increase on the 7th day, decrease on the 14th and 28th days) have confirmed, although only the decreases, own results obtained earlier [26]. In the latter studies [26] on rabbits immunized with *Ch. psittaci* – strain 6BC, decreases in LZM amounts were recorded on the 14th, 21st, 35th and 56th days and a lowered activity of LZM on the 7th, 14th and 28th days of the studies. However, it should be added that the studied failed to demonstrate that the employed strain 6BC of *Chl. psittaci* induced increase in the amount and activity of LZM, which has been documented by the present experiment. It can be assumed that the strain probably induces alterations in the amount and activity of LZM but that they are not always equally intense. Nevertheless, the presented results indicate that the presently studied indices of non-specific humoral immunity, such as MPO and LZM, represent elements of anti-chlamydial resistance while intensity and duration of their manifestation reflect the type and strain of the bacteria. Participation and role of the non-specific humoral

immunity in anti-chlamydial resistance have also been confirmed by observations on other indices of non-specific immunity, such as peroxidase [4], acid phosphatase, β -glucuronidase, cathepsin D [7], IFN, TNF- α [9, 10, 13] as well as IL-1, IL-6, IL-12 [8, 10–12]. The studies [4, 7–13] demonstrate that infection or immunisation of mammals with *Chlamydia* spp. or *Chlamydophila* spp. induces increase in the level or activity of substances which inhibit bacterial proliferation or lead to their destruction in cells of the macroorganism. They also prove that destruction of *Chlamydia* spp. and *Chlamydophila* spp. engages not only oxygen-independent cidal systems such as, e.g., LZM or interleukins but also the oxygen-dependent systems like, e.g., MPO, peroxidase and reactive forms of oxygen. Involvement of the indices in defense against the intracellularly parasiting bacteria has been confirmed by observations on mice experimentally infected with *Mycobacterium tuberculosis* [27], in which the destruction processes targeted at the intracellular parasiting bacteria were found to be linked to substances such as TNF- α , IL-1, IL-6 and IL-8, as well as to reactive forms of oxygen and nitrogen. Thus, the results of earlier [26] and present investigations as well as results of other authors [4, 7–13, 26] prove that factors of non-specific humoral immunity represent a significant protective element against intracellularly parasiting bacteria since act, i.a., directly at the site in which the microbes are present.

On the other hand, in evaluation of the results on serum immunoglobulin levels it should be noted that the presently examined strain 6BC of *Chl. psittaci* has manifested its effect in the dynamic evolution of alterations even if it has induced no significant changes in total immunoglobulin levels or IgG levels. This has also been observed in the cattle naturally infected with *Ch. psittaci*, in which augmented amounts of serum IgG₁ levels and lower IgM have been noted [14]. According to the authors, the serum immunoglobulins play principal role in protection against the infection with *Ch. psittaci*. The role of immunoglobulins in humans, guinea pigs, mice and goats, infected naturally or experimentally (this pertains laboratory animals) with *Chlamydia trachomatis* or *Chlamydophila psittaci* (earlier: *Chlamydia psittaci*) has been described also by other authors [16–19, 28, 29], who have detected also elevated levels of serum immunoglobulins (of G, A and M classes) [16–19] and of secretory sIgA (in milk) [19]. The results [16–19, 28, 29] find practical confirmation in diagnoses of chlamydioses in humans [28, 29], in whom the antibodies, particularly those of IgG and IgM classes, represent a specific diagnostic element of *Chlamydia tra-*

chomatis infection. Involvement of serum immunoglobulins in infections with intracellularly parasiting bacteria is documented by the elevated serum IgG in mice experimentally infected with *Listeria* sp., *Orientia tsutsugamushi* and *Ehrlichia chaffeensis* – the intracellular bacteria [27].

Evaluating the presently recorded relatively insignificant alterations in haematological patterns (elevated level of neutrophilic granulocytes and decreased content of basophilic granulocytes and of monocytes) in rabbits immunized with *Chl. psittaci* – strain 6BC, it should be disclosed that they confirm the pattern typical for the type of immunization in rabbits [30]. A similar pattern, manifested also by slight changes in leukocyte number, has been documented in patients with ornitosis [31, 32], in whom leukocyte levels increased at the start of the disease and decreased subsequently. The changes in white blood cells analogous to those described in patients with ornitosis have been described in sheep naturally infected with chlamydia [33], while in the naturally infected cattle only augmented leukocyte levels have been noted [34]. It should also be detected that the values of haematological parameters documented for rabbits immunized with *Chl. psittaci* – strain 6BC did not diverge far from physiological values [35], which may suggest that immunization of rabbits with those bacteria induces only

slight and relatively non-specific alterations in the parameters.

Analysis of positive antibody titres noted in the studied rabbits starting at 49th day after immunization with *Chl. psittaci* corroborates observations of other authors [30, 36], who demonstrated the antibodies in the 5th week after immunization of rabbits with *Chl. psittaci* [30] and after 8 weeks in sheep with abortion due to *Chl. abortus* infection [36]. Specific antibodies detected already on the 7th day after immunization of rabbits with *Chl. psittaci* have provided evidence for stimulation of lymphocytes B, which seems to confirm the general principle that in infection or immunization with bacterial antigens serum Ig synthesis develops after 5–7–10 days [37].

Among five examined indices of immunity and eight haematological parameters, immunization of rabbits with *Chl. psittaci* – strain 6BC induced significant alterations only in the amount and activity of LZM and basophilic and neutrophilic granulocytes and monocytes, involving periodically augmented and periodically decreased levels. The recorded alterations, mainly those in immunological indices, appearing 6 weeks before titres of specific anti-*Chlamydia* spp. antibodies could be detected, may indicate that the heretofore tested immune parameters should be estimated in such infections.

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